patterns are established in three-dimensional tissues including peripheral organs and the central nervous system (CNS). We study the mechanisms underlying the patterning of blood vessels in CNS using early spinal cord as a model. In chicken E4 embryos, endothelial cells of somitic origin penetrate into the spinal cord through the ventral edge. Subsequently, forming vessels extend dorsally along determined paths. We found that these paths coincide with the interface between the layer of progenitor cells (medial) and the layer of differentiated neurons and glial cells (lateral). Since the blood vessels do not invade the progenitor layer, we reasoned that the progenitor layer elicits inhibitory influence on the blood vessel formation. To test this, we experimentally promoted neural/glial differentiation in the progenitor territory. When the progenitor layer was electroporated with Pax2, a transcription factor known to promote glial cell differentiation, the cells underwent precocious differentiation. Remarkably, in the Pax2-electroporated region, an ectopic formation of blood vessels was observed. These observations support the idea that the blood vessel patterning in CNS is regulated by local environment, where neural progenitor cells appear to prevent the vessel formation. We will discuss the relationship between vascular patterning and neural differentiation.

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Program/Abstract # 390
Regulation of the CamkII node determines proliferative potential of growth plate chondrocytes

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For tissues that develop throughout embryogenesis and into postnatal life, the generation of differentiated cells to promote tissue growth is at odds with the requirement to maintain the stem cell/progenitor cell population to preserve future growth potential. In the growth plate cartilage, this balance is achieved in part by establishing a proliferative phase that amplifies the number of progenitor cells prior to terminal differentiation into hypertrophic chondrocytes. Upregulation of endogenous calcium/calmodulin-dependent protein kinase II (CamkII) activity in chondrocytes promotes proliferative phase exit and is required for chondrocyte hypertrophy. CamkII activity in chondrocytes is under negative regulation by the Wnt and the Pthrp signaling pathways and release of repression activates multiple independent, dosage-sensitive effector pathways that compose the terminal differentiation (hypertrophic) program. In doing so, CamkII activity places strict limits on the growth potential of proliferative chondrocytes. We present an integrated model for the regulation of proliferative potential that has important implications for studies of adult stem cells.

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Program/Abstract # 392
Epicardial spindle orientation controls cell entry into the myocardium

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During heart morphogenesis, epicardial cells undergo an epithelial to mesenchymal transition (EMT) and migrate into the subepicardium. The cellular signals controlling this process are poorly understood. Here, we show that epicardial cells exhibit two distinct mitotic spindle orientations, directed either parallel or perpendicular to the basement membrane. Parallel division, in which the mitotic spindle is ~180° to the basement membrane, results in epicardial cell expansion, while cells undergoing perpendicular cell divisions subsequently enter the myocardium. We found that loss of β-catenin led to a disruption in adherens junctions, randomization of mitotic spindle orientation, and reduced cell entry into the myocardium. Surprisingly, loss of β-catenin correlated with the loss of Numb asymmetric localization to adherens junction and basal domain of epicardial cells. Loss of Numb and Numblike in the epicardium disrupted adherens junctions, led to randomized mitotic spindle orientations, and also had reduced numbers of epicardial cells entering the myocardium. Taken together, these data suggest that directed mitotic spindle orientation contributes to epicardial EMT and implicates a junctional complex of β-catenin and Numb in the regulation of spindle orientation.

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